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240 250 260 270 280 290 300 310
| | | | | | |
GGCCACGGCCATTGCCAGACCGGTGACTGCGGGGTCTCCTTGCTGCACGGCTACGGCTCCCTCCCGACACCTC
G H G H C O T G D C G G L L A C T A Y G S P P D T L

320 330 340 350 360 370 380 390
 GCAGAAATTCGCCCTGAACCCAGTACGCCGCCAGGACTTCTACGACATCTCCCTCGTCGACGGCTTCAACATCCCCCATG
 A E F A L N Q Y A G Q D F Y D I S L V D G F N I P M

400 410 420 430 440 450 460
 GACTTCTCCCGACGTCCGGAATTGCCACGACATCCGGTGACCGCGGACATCAACGGTCAAGTCCCGCGGAGCTG
 D F S P T S G N C H D I R C T A D I N G Q C P A E L

470 480 490 500 510 520 530 540
 AAGCACCCCGGGGTGTAACAACCCGTGCACCGGTGTTCAAGACCAATGAGTACTGCTGCACTTCGGGAGGCTGTGGG
 K A P G G C N N P C T V F K T N E Y C C T S G G C G

550 560 570 580 590 600 610 620
 CCCACGGACTATCCAAAGTTTTC AAGCAGAGGTGCCCTGATCGGTACAGTTACCCCAAGGATGACGCTACCAAGCACT
 P T D Y S K F F K Q R C P D A Y S Y P K D D A T S T

630 640 650 660 670 680 690 700
 TTTACTTGTCGCCAGTGGGGCTGATTACAGGGTTGTGTCTGCCCTTGATCGAGCTTACTCAGATGTTGTGTGAGCAAT
 F T C P S G A D Y R V V F C P *

710 720 730 740 750 760 770
 CAACTATGGTTAATTGTACGTAGCTCATTAAAGAACCGGAATAAGGTCCGATGTAAGCTCTACTTGAGC

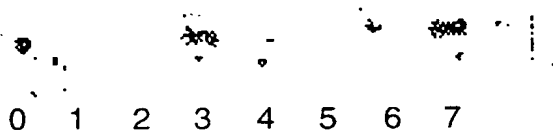
FIG. 1 CONT'D

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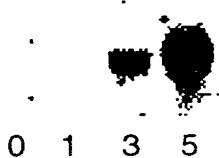
FIG. 2
Similarity of AoPRT-L to other PR-5 Group Proteins

Protein	Cellular Location	pI	<u>Similarity or Identity</u> to AoPRT-L	Percentage
AoPRT-L	Extracellular	4.9	100	100
Osmotin	Vacuolar	7.5	89	77
Tobacco Osmotin-like	Vacuolar	7.5	89	77
Tobacco Thaumatin-like	Extracellular	5.2	80	80
Tomato NP24	Vacuolar	7.8	78	65
Thaumatin	Cytoplasmic	12.0	76	63
Potato Osmotin-like	?	6.1	76	62
Rice Thaumatin-like	?	5.0	70	53
Wheat Thaumatin-like	Extracellular	4.5	68	49
Barley Thaumatin-like	Extracellular	4.2	67	49

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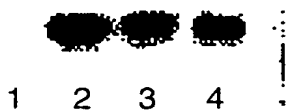
FIG.3a**Induction of AoPRT-L following cell isolation**

Time after isolation (days)

FIG.3b**Induction in etiolated seedlings by wounding**

Time after wounding (days)

Explant length 5mm

FIG.3c**Induction of AoPRT-L in whole plants by SA****Time course of induction following foliar application of 1mM SA to whole plants**

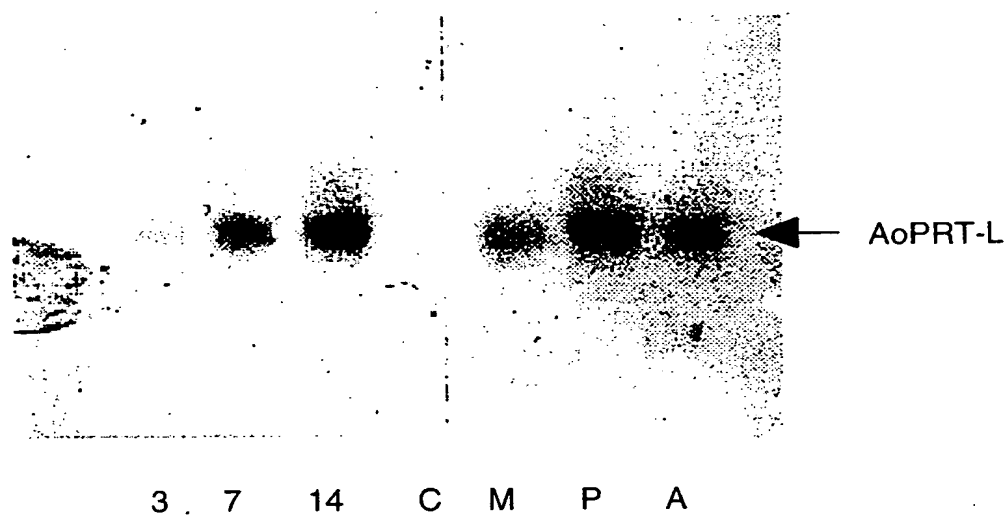
Time after application (days)

- 1; Water treated
- 2; 3 days after foliar spraying with 1mM SA
- 3; 3 days after continuous root feeding with 1mM SA
- 4; 3 days after initial root feeding with 1mM SA

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FIG.4

**AoPRT-L Expression in Asparagus seedlings
infected with *Stemphyllium versicarium***



Figures (3, 7 & 14) indicate days after symptom development

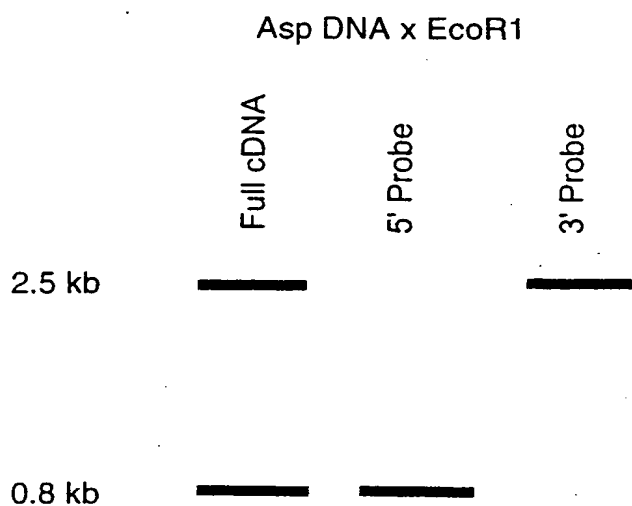
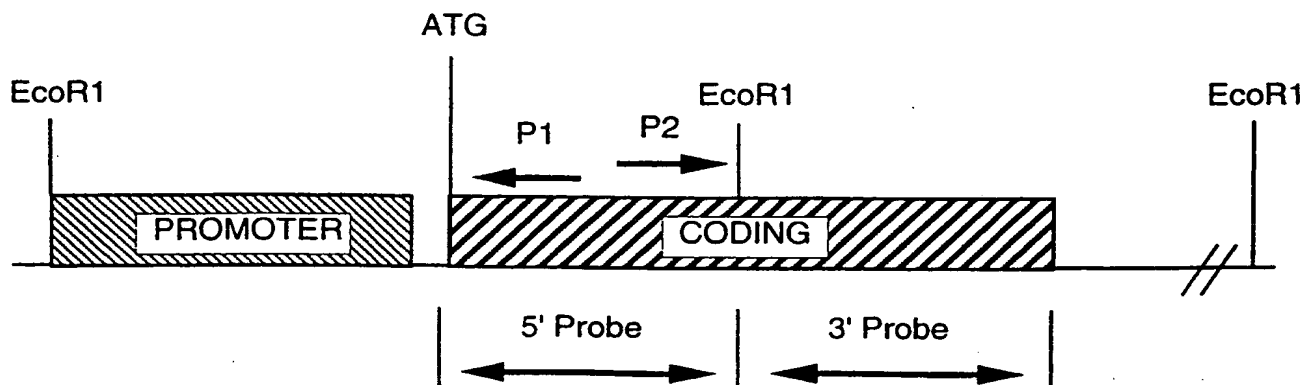
C - uninfected Asparagus

M - Infected region (day 14)

P - Pigmented region (day 14)

A - Asymptomatic region (day 14)

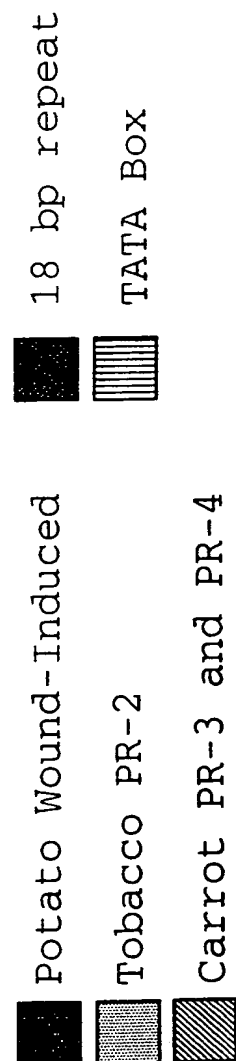
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FIG. 5**IPCR Strategy****Southern Analysis****Primer Design**

AoPRT-L Promoter sequence : Similarities with other Defence Genes

-472 GAATTCTTAT TCGGACCTGA CTCTCTTGTT GTGCTGCCGA GGTGCTGTCTG
 -422 AAATTTCTGT TCGCACAAAC ATACTGGTCC TTGCTTGATT TGACAGTTCC
 -372 AATAATTATT TCCATGTCAT GAGAGAAGCA CATGACTAAA GTAATTAGCT
 -322 TAATCCCCCTA AACTCAATA CAAACGAGAT GACACATCCA CAGAAAAAAT
 -272 TCTAATTAGT CTTTGCGTGT AGAAATTGGA AACTGAATAC CTACATTAAT
 -222 TACAACCTTT GCAATAATAA TATAAGAAA GTTCTAACAT GAAGACTAGT
 -172 TCTAACATGA AGACTAGTCC ACGAACTCGT ACCTTATTCC ACAAAGGCTT
 -122 AGACTTTCCA CAAATCGAGA TTATCCCATG GACTGATGA CACCATCCAA
 -72 ATTATCCCTA TAAATACCTG CCCATTCCCC TCCTCCAGAC TCATCTAACT
 -22 CAAAAACAAC ACACAACCAA TCATG

FIG. 6



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pIPCR-TA

PCR using 5' and 3' primers
Clone into pJIT60 using KpnI and PstI

**p22-JIT60**

Clone in GUS(INT) using BamHI and EcoRI

**p22-GUS(INT) JIT60**

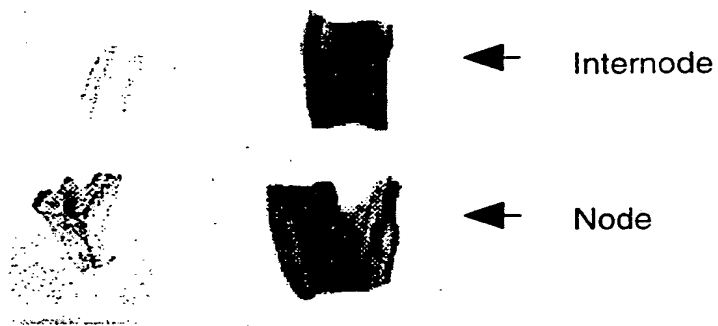
Cut with KpnI and XhoI and clone into KpnI and SalI cut pBin19

**p22-GUS(INT) Bin19****FIG. 7**

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FIG.8

**Histochemical localisation of GUS activity in
untreated stems from transgenic tobacco
harbouring AoPRT-L-GUS or PR-1a-GUS**

AoPRT-L-GUS**PR-1a-GUS**

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FIG. 9

AoPRT-L-GUS Expression in TMV-infected Tobacco

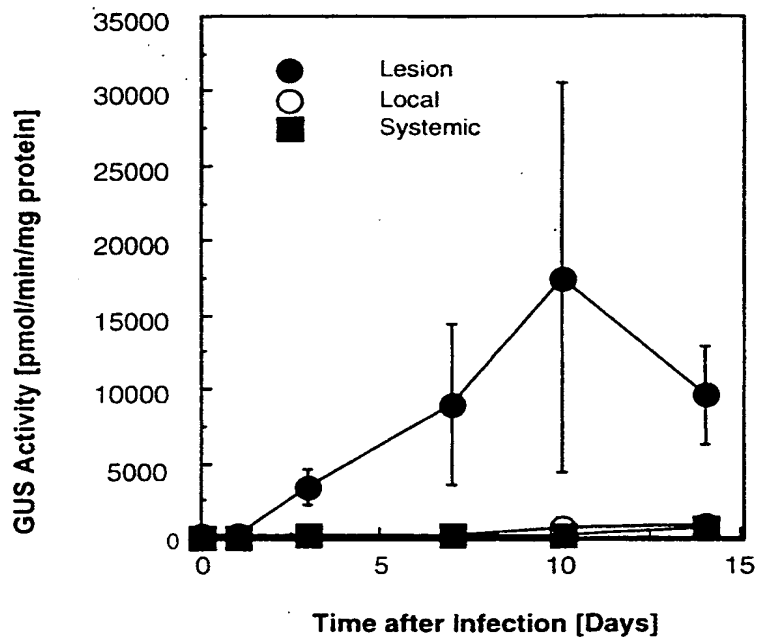
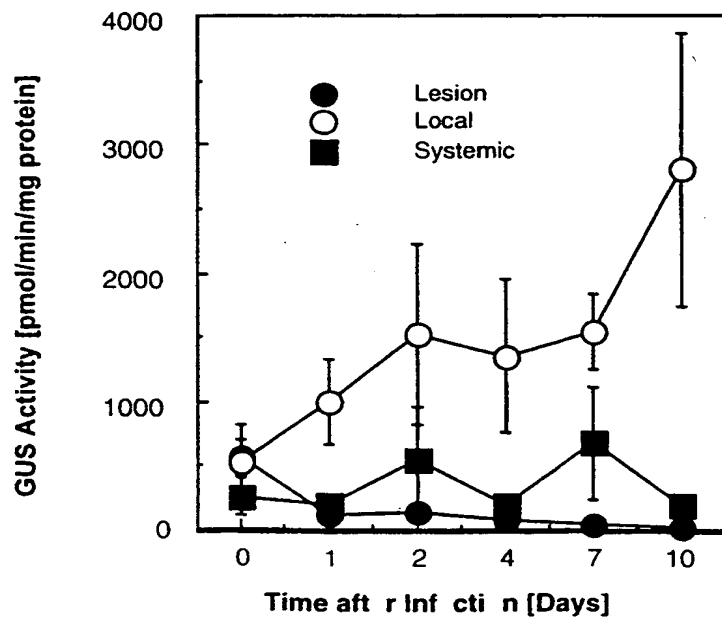
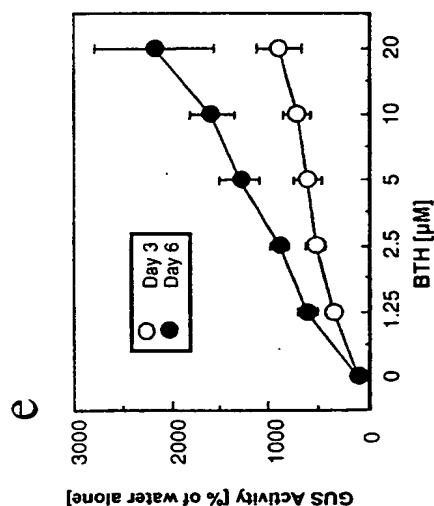
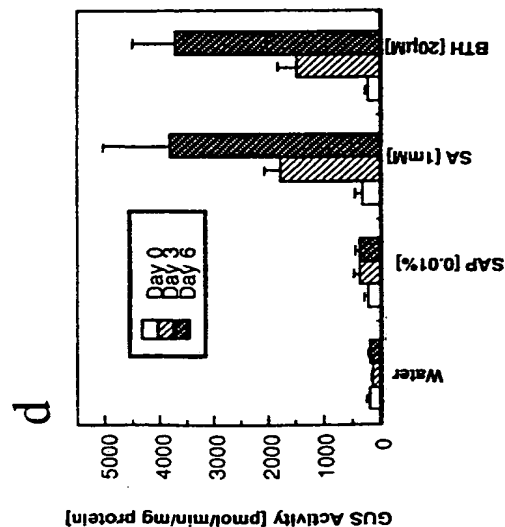
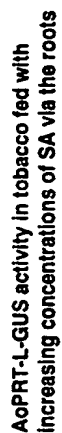
AoPRT-L-GUS Expression in Tobacco infected with *Pseudomonas syringae* pathovar *phaseolicola*



FIG. 10



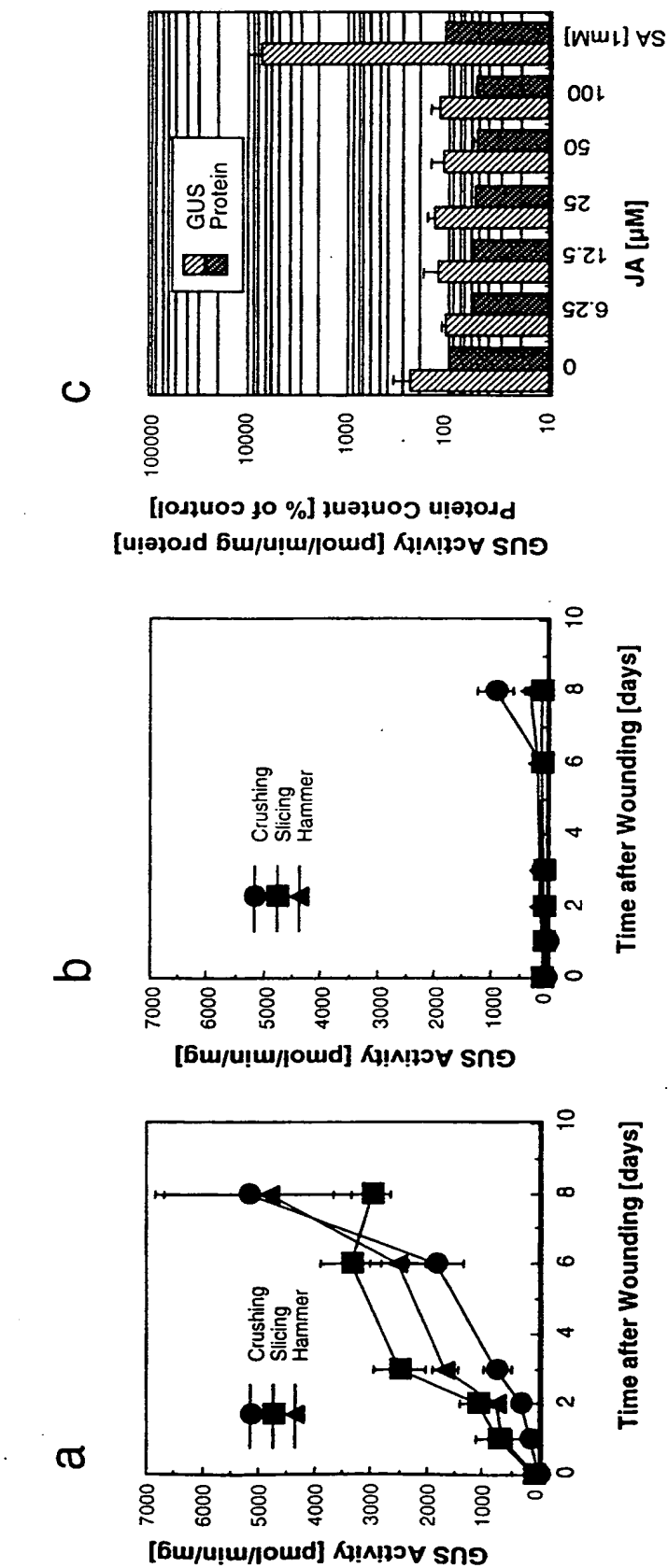
AoPRT-L-GUS activity in tobacco leaf discs treated with increasing concentrations of BTH



**AoPRT-L-GUS activity in tobacco plants
sprayed with SA or BTH (diluted in 0.01%
sapogenat - SAP)**

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FIG. 11
Effects of wounding and JA on GUS expression in transgenic plants



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AoPRT-L-GUS Expression Following Water Stress

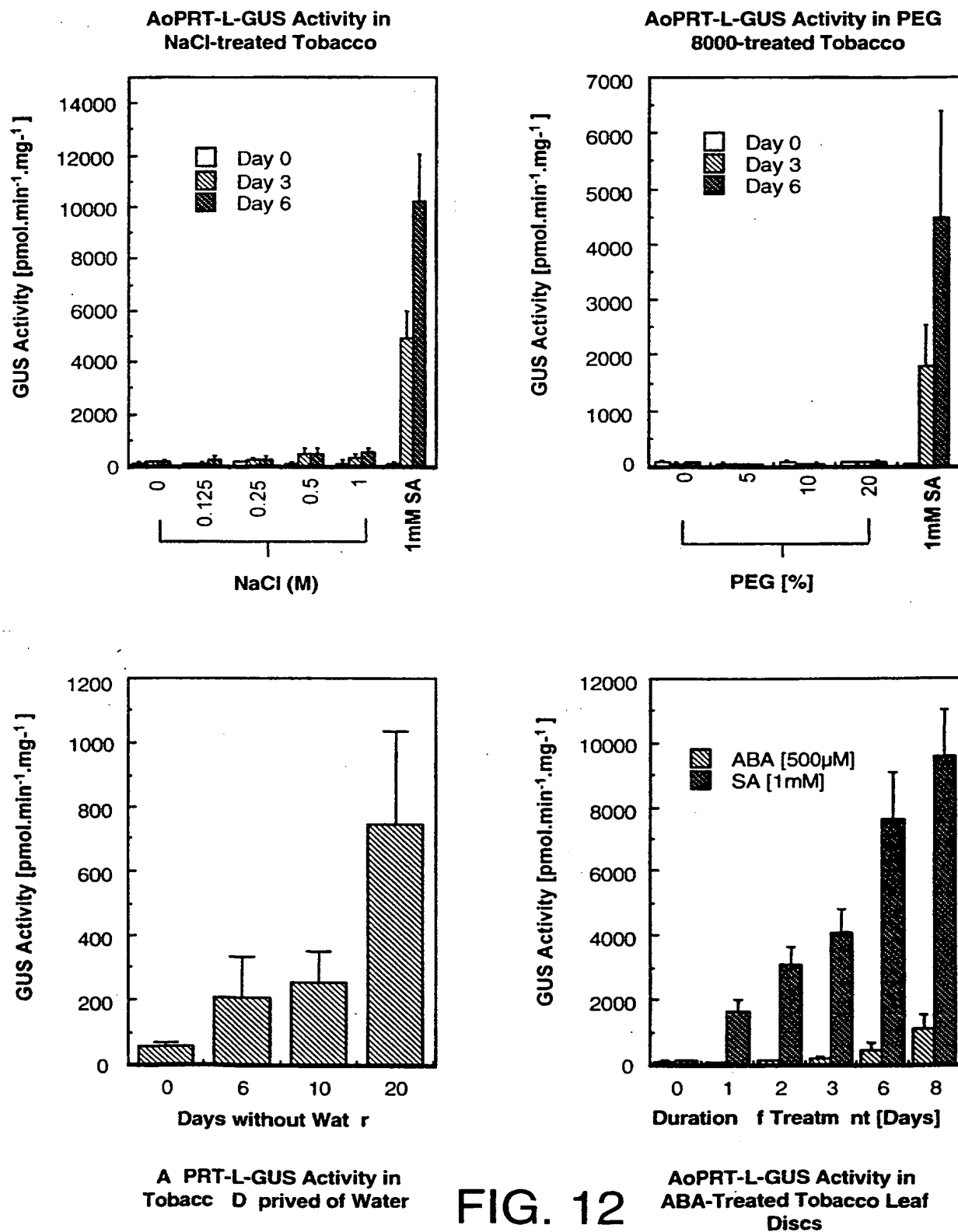


FIG. 12

FIG. 13 AoPRT-L-GUS Expression following Oxidative Stress

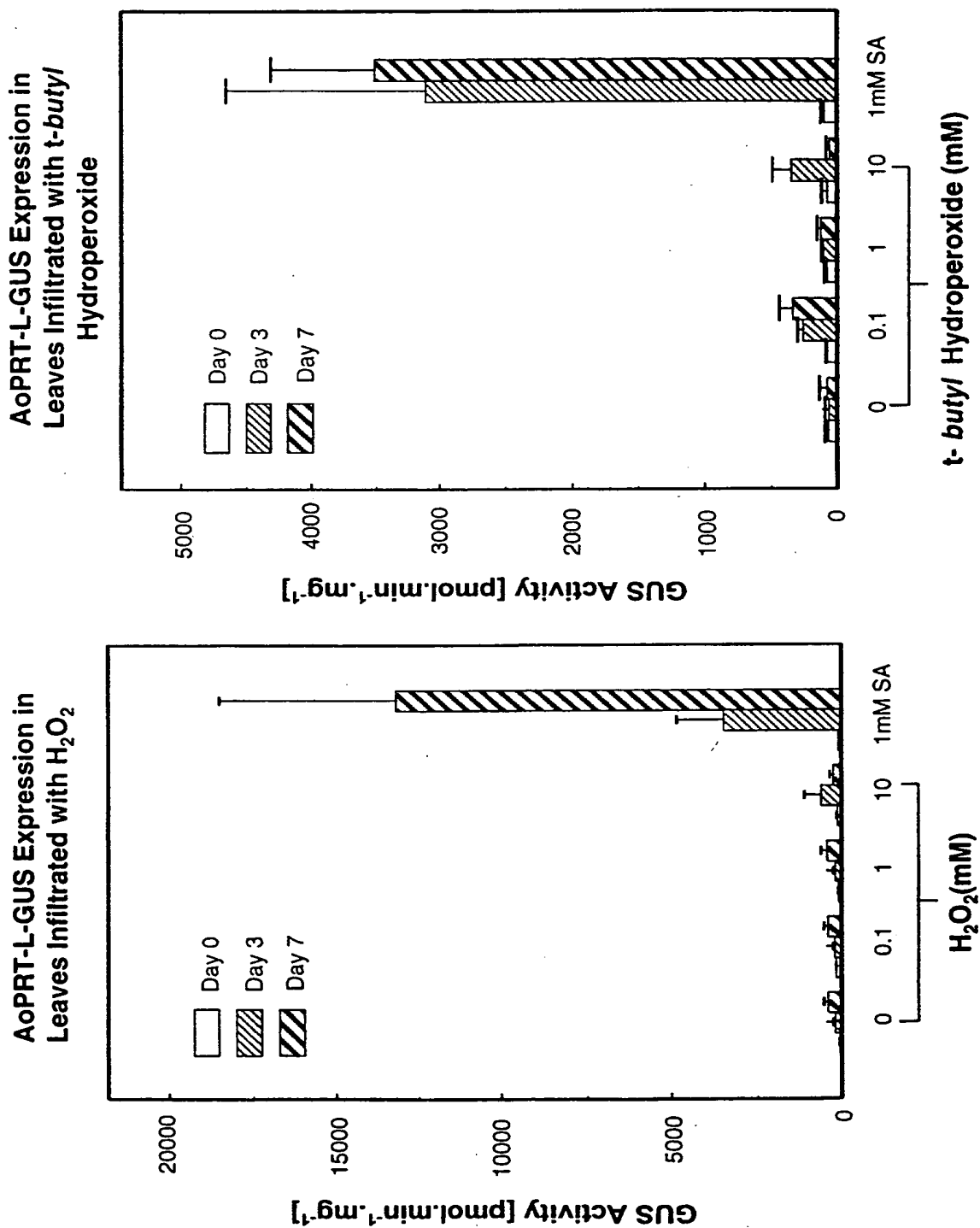
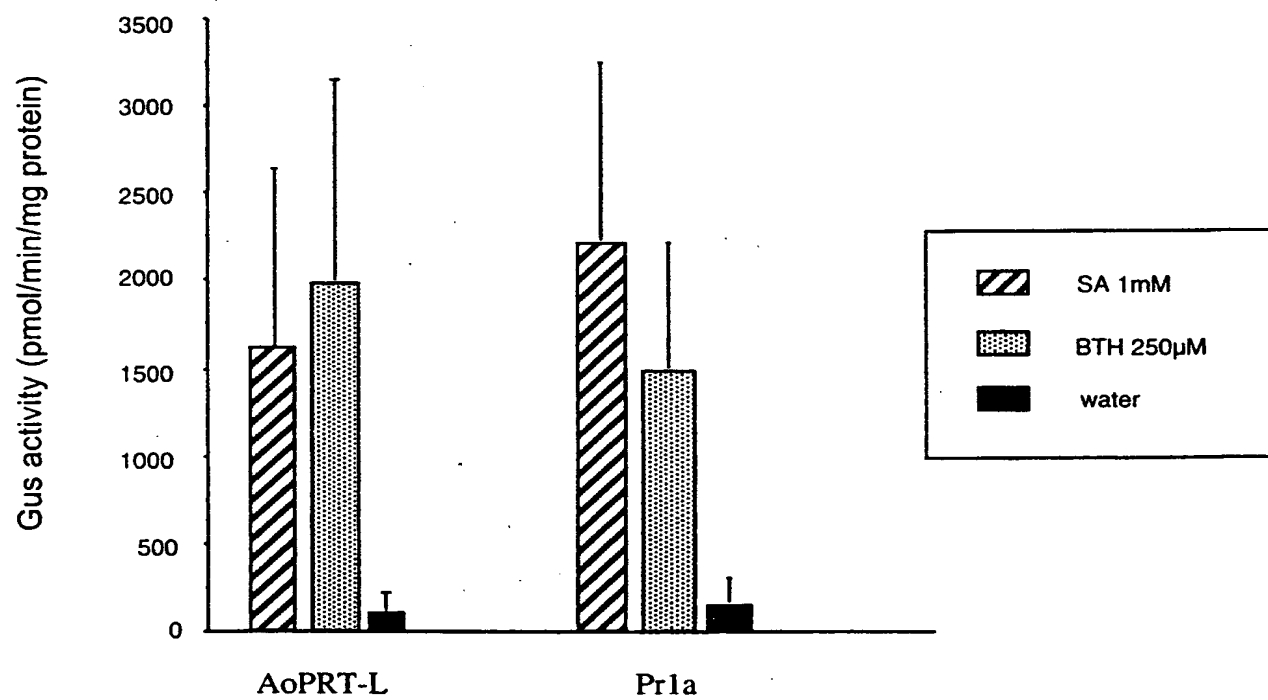
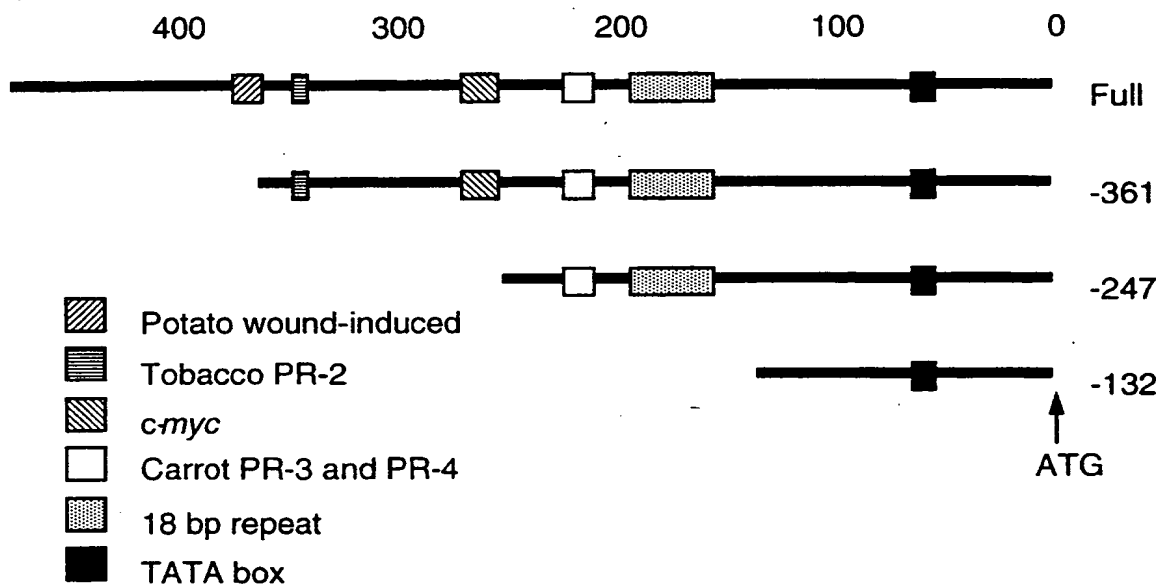
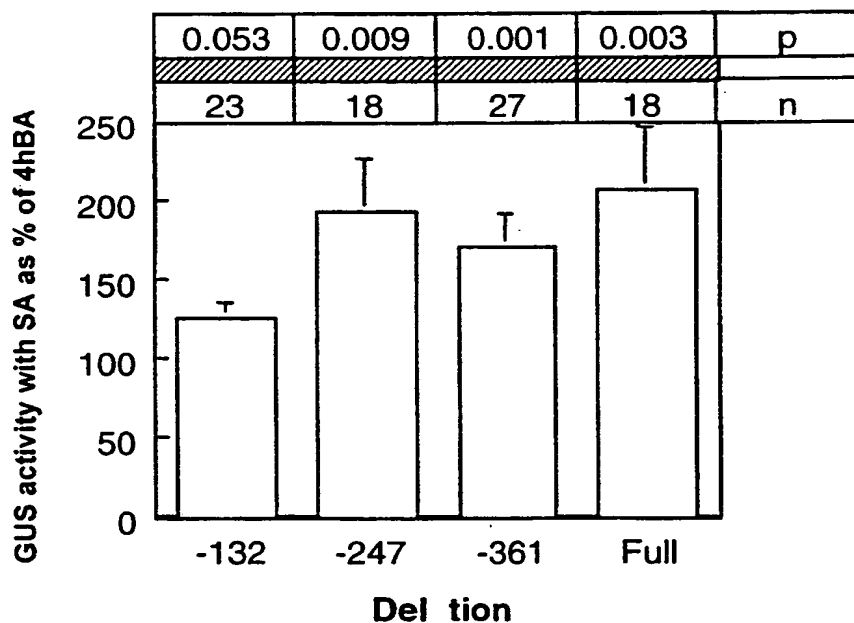


FIG. 14

AoPRT-L-GUS and Pr1a-Gus expression after SA or BTH induction in Brassica napus leaves



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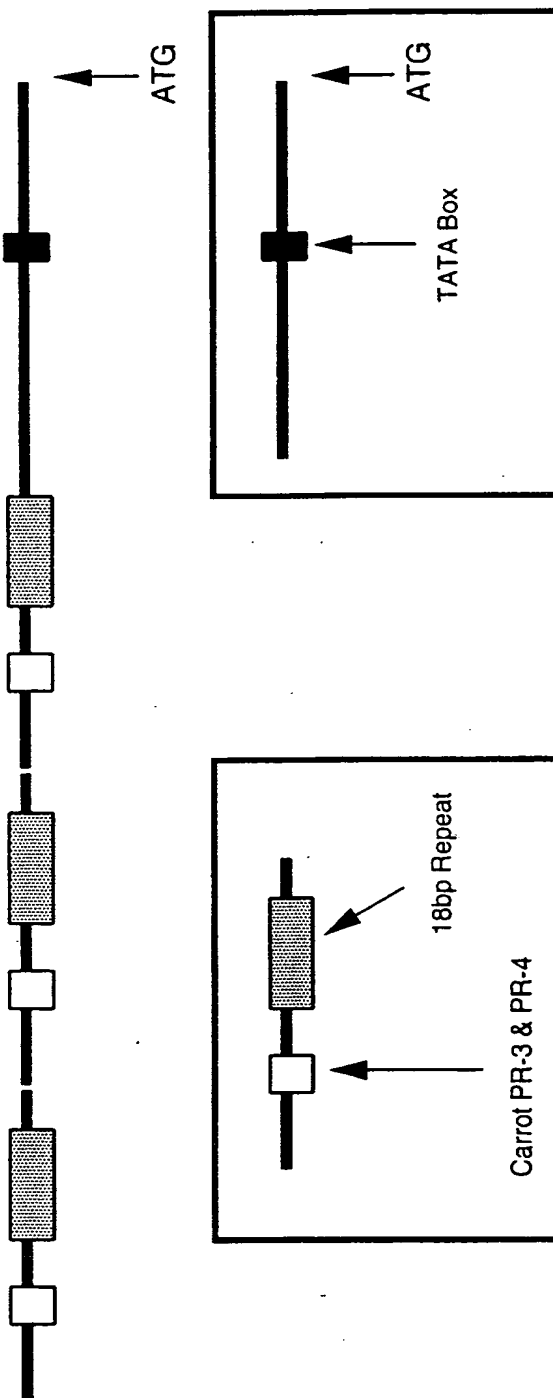
FIG. 15a AoPRT-L promoter deletions**FIG. 15b SA-responsiveness of AoPRT-L promoter deletion-GUS constructs in T0 transgenic tobacco plants**

n - number of individual transformants

p - probability that activity with SA is not different to activity with control-treatment (Wilcoxon signed rank test)

Multimerised AoPRT-Lx3 SA-responsive promoter

The -247 to -133 putative SA-responsive region cloned into pJIT-60 GUS (INT) containing the AoPRT-L minimal promoter (-132 to -1)



AoPRT-L-min

(-132 to -1)

AoPRT-L-SA

(-247 to -133)

FIG. 15C

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FIG. 16
schematic diagram of plasmid pGB24

